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Sepsis is a disease caused by invasive infection, which causes systemic inflammation and leads to greater than four million deaths worldwide. About 50% of critically ill children suffering from sepsis in the U.S. receive a blood transfusion during their stay in the ICU. Recent research has led to mounting evidence indicating that RBC transfusions in patients with sepsis are associated with altered immunity and worsened outcome. Transfusion in sepsis is associated with dysregulated immunity. During transfusion, some donor RBCs lyse, generating microparticles and free hemoglobin (Hb), which then release cell-free heme (CFH) into plasma. In septic adults, increased plasma Hb and depletion of the CFH scavenger, hemopexin (Hpx), are associated with increased mortality. Recent work (in animals) indicates that CFH in plasma may directly activate TLR4, the LPS receptor. As such, heme release in excess of plasma binding capacity has the potential to worsened inflammation and may influence survival.

We used a murine cecal ligation, puncture (CLP) model of sepsis, and mimicked RBC transfusion via exogenous, commercially obtained heme. After initial LD50 heme studies without sepsis, we conducted heme dose response preliminary studies in B6/C57 strain mice and CLP (n=29) for 72 hours. After CLP (50% ligation-21G single puncture), each mouse was either injected via tail vein with PBS (control) or heme (10 ÂμM, 50ÂμM, or 500ÂμM; n=9, 8, 10, 2 respectively). At 2 hours and 24 hours. post-CLP, heme measurement were obtained and each animal was re-dosed with PBS (control) or respective heme.

All heme administered animals died by 61 hours (mean 40.4hÂ±12.5) in a dose dependent fashion, while controls had a 45% survival. Circulating heme levels were lowest among controls. Spleen, liver, and lung tissue samples showed an increase in apoptosis and tissue denudation as dosage of heme was increased.

We demonstrate that three separate doses of heme given in a CLP model of murine sepsis has 100% lethality. Future studies will carefully investigate innate immune phenotype during sepsis evolution and evaluate the utilization and timing of administration of CFH and potentially scavengers (i.e., Hpx) to improve survival.